

## Electrophysiological Techniques for Sea Lion Population-level Audiometry

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Award Number: N0001409WR20202

<http://www.spawar.navy.mil/sandiego/technology/mammals/>

### LONG-TERM GOALS

Apply electrophysiological techniques to perform large-scale auditory testing of Navy sea lions

### OBJECTIVES

The objectives of this project are to (1) modify electrophysiological techniques previously developed for dolphins to assess hearing sensitivity in California sea lions (*Zalophus californianus*), (2) benchmark the techniques using behavioral hearing data from the same individual, and (3) apply the techniques to measure the hearing sensitivity of all available Navy sea lions.

### APPROACH

ONR funding for FY09 was in support of a proposed two-year SSC Pacific In-House Laboratory Independent Research (ILIR) project consisting of two main tasks. Task I consists of measuring in-air and underwater behavioral hearing thresholds and comparing to in-air AEP thresholds obtained from the same individual. During task II, AEPs would be used to assess the hearing of all available MMS sea lions.

In task I, AEP thresholds will be repeatedly tested on a single sea lion under sevoflurane anesthesia. Behavioral hearing thresholds will be obtained underwater and in air for the same individual and will be used to benchmark the AEP thresholds to those obtained behaviorally. Due to the robust nature of auditory evoked potentials obtained in dolphins and differences in auditory processing capabilities relative to the sea lion, the AEP techniques used for dolphins will not be directly transferrable to sea lions. Subcutaneous electrodes will be required to record evoked responses in sea lions and different stimulus parameters will be required to obtain optimal evoked responses for the estimation of hearing sensitivity. Therefore, during task I effort will be directed toward optimizing electrode placement and stimulus parameters.

During task II, the AEP system will be used to assess the hearing of all available MMS sea lions utilizing the techniques refined during task I. The current inventory consists of 29 animals and all animals will be tested, subject to availability.

Report Documentation Page				Form Approved OMB No. 0704-0188	
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1. REPORT DATE <b>2009</b>		2. REPORT TYPE		3. DATES COVERED <b>00-00-2009 to 00-00-2009</b>	
4. TITLE AND SUBTITLE <b>Electrophysiological Techniques For Sea Lion Population-Level Audiometry</b>				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <b>Space and Naval Warfare Systems Center Pacific,Biosciences Division, Code 71510,53560 Hull Street,San Diego,CA,92152</b>				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release; distribution unlimited</b>					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT <b>The objectives of this project are to (1) modify electrophysiological techniques previously developed for dolphins to assess hearing sensitivity in California sea lions (Zalophus californianus), (2) benchmark the techniques using behavioral hearing data from the same individual, and (3) apply the techniques to measure the hearing sensitivity of all available Navy sea lions.</b>					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT <b>Same as Report (SAR)</b>	18. NUMBER OF PAGES <b>4</b>	19a. NAME OF RESPONSIBLE PERSON
a. REPORT <b>unclassified</b>	b. ABSTRACT <b>unclassified</b>	c. THIS PAGE <b>unclassified</b>			

## WORK COMPLETED

### *Behavioral hearing tests*

Our initial sea lion subject (SLD, Fig. 1) was a 7-year old male with no prior experience with cooperative psychophysical testing. The subject was first desensitized to the test environment and headphones, then trained to produce a vocal response to audible hearing test tones. After the response rate neared 100%, the tone amplitudes were adjusted in staircase fashion to approach thresholds. After several weeks of effort to condition the subject to respond to increasingly lower tone amplitudes, we began to suspect that the subject possessed some pre-existing hearing loss. Eventually, comparison of AEP data from this subject to that of two other sea lions convinced us that this subject did not have “normal” hearing and was thus not an appropriate subject for the behavioral/AEP data comparison. Subsequently, we obtained a new sea lion subject, a 1-year old male (JFN). This animal had stranded multiple times and was classified as non-releasable. The animal had no experience in behavioral conditioning or psychophysical testing. At present, we have trained this subject to station on the test apparatus and produce a vocal response to hearing test tones of various frequencies.

### *Evoked potential hearing tests*

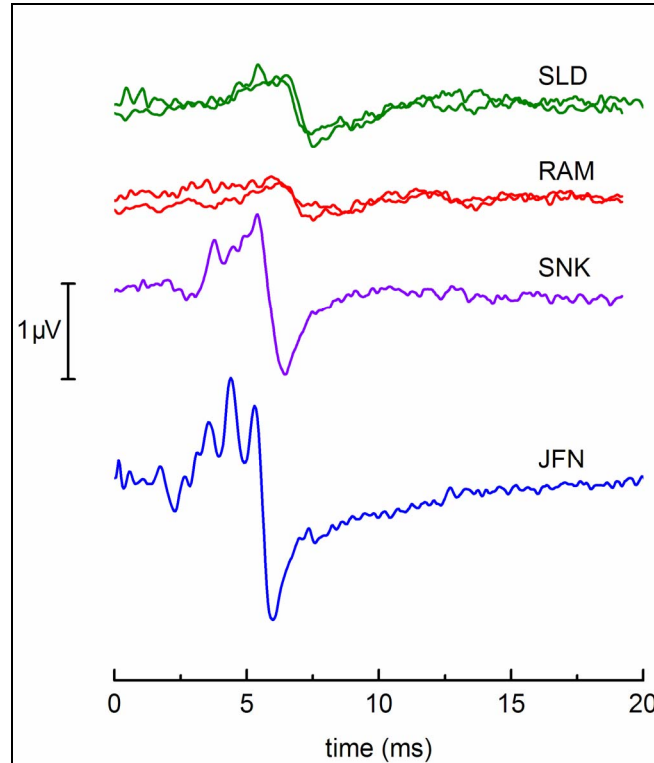
We measured AEPs in four sea lions: SLD, JFN, RAM (a 25-year old male Navy Marine Mammal Systems sea lion), and SNK (a 3-year old male Navy Marine Mammal Systems sea lion). Initially, calibration procedures and optimal electrode positions were determined. Next click-evoked potentials were measured using 50–100  $\mu$ s stimuli. In SLD, SNK, and JFN, auditory steady state potentials (ASSRs) were measured in response to single sinusoidal amplitude modulated tones. In JFN, auditory thresholds were measured in response to both single and multiple, simultaneously presented, SAM tones.



*Figure 1. The sea lion SLD wearing headphones during a behavioral hearing test.*

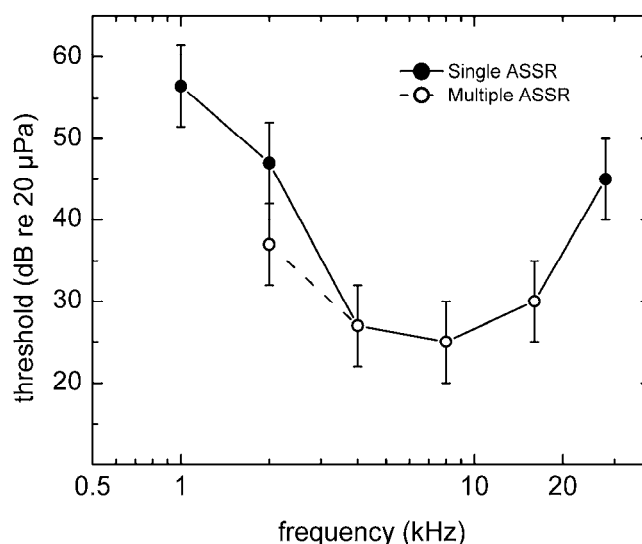
## RESULTS

Figure 2 shows click-evoked potentials recorded from each subject in response to a 100  $\mu$ s click at  $\sim 95$  dB re 20  $\mu$ Pa (peSPL) sound stimulus. The differences in amplitudes and latencies across these four subjects suggest significant hearing loss in SLD and RAM compared to JFN and SNK. Although this result could be anticipated for RAM (because of his age), we did not anticipate seeing significant hearing loss in SLD since he is only 7 y.



**Figure 2. Click-evoked potentials recorded in four sea lions. All four waveforms exhibit similar positive and negative waves near 5–7 ms; however, the smaller amplitude and increased latency for SLD and RAM suggest significant hearing loss compared to SNK and JFN. This observation was supported by behavioral results from SLD, who did not reliably respond to tones with SPLs below  $\sim 70$  dB re 20  $\mu$ Pa.**

Figure 3 shows AEP hearing thresholds as a function of frequency for JFN. The audiogram shows good hearing ability for JFN up to  $\sim 30$  kHz. There was also good agreement between the thresholds measured using single SAM stimuli and multiple, simultaneous SAM stimuli. This is important, since large scale hearing screening of Navy sea lions would benefit from the increase in speed provided by the multiple ASSR approach.



**Figure 3. Auditory thresholds in JFN measured with single and multiple SAM stimuli. Best sensitivity was between 4 and 16 kHz, where thresholds were ~25 dB re 20 µPa. The upper limit of hearing was approximately 30 kHz. There was good agreement between the thresholds measured using the single and multiple ASSR techniques.**

## IMPACT/APPLICATIONS

The techniques developed under this project will be directly applied to hearing screening of Navy Marine Mammal Systems sea lions to insure that the effectiveness of sea lion-based systems is not compromised due to poor hearing. These techniques may also be applied to testing wild pinnipeds, with the increase in speed and optimization of techniques allowing greater probability of success and/or reduced time required for testing of wild or rehabilitating animals.

## RELATED PROJECTS

“Temporary threshold shift (TTS) in odontocetes in response to multiple airgun impulses,” is a related project funded by the International Association of Oil and Gas Producers, Joint Industry Project (JIP). This effort employs techniques and equipment for behavioral and AEP hearing tests developed under previous ONR efforts.

“Auditory weighting functions and frequency-dependent effects of sound in bottlenose dolphins (*Tursiops truncatus*),” is a related project funded by ONR (N0001409WX20358). This effort employs techniques and equipment for behavioral and AEP hearing tests developed under previous ONR efforts.